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Durand L., Guinebretière M.H., Carlin F., André S., Remize F., Planchon S. (2013) "**Genetic and phenotypic diversity of two highly thermoresistant species responsible for Low Acid Canned Foods spoilage**" (Communication orale). Microbial Spoilers in Food 2013, Quimper, 1, 2 et 3 juillet.

Genetic and phenotypic diversity of two highly thermoresistant species responsible for Low Acid Canned Foods spoilage

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Introduction: Two thermophilic bacteria, the anaerobe *Moorella thermoacetica* and the aerobe *Geobacillus stearothermophilus*, are responsible for 75% of low acid canned foods spoilage at high incubation temperatures (55°C) . These bacteria are largely present in the environment and consequently contaminate vegetable and prepared meals processing lines. Both species form Highly Heat Resistant Spores (HHRS) which can withstand the sterilization process. Few studies concerning genetic diversity are available on these species. Improved knowledge of these species concerning genetic and physiologic properties, will lead us to a better understanding of their ecology and a more accurate strategy for control them in canned foods.

Material and methods: A total of 140 *G. stearothermophilus* and 94 *M. thermoacetica* isolates, collected from spoiled canned foods after incubation at 55°C, over a period of ten years, were used in this study. References strains (5 DSMZ and 1 ATCC) were added. A M13-PCR based method was selected and applied to type isolates for each species. Different groups were obtained by electrophoretic profiles analysis with the Biogene software (Bio Profil V99.04). The phenotypes of selected isolates (one per group) will be further characterized for growth and sporulation temperature and pH, and spore heat resistance.

Results: Nineteen groups were obtained for each of the species *G. stearothermophilus* and *M. thermoacetica* in the selected conditions. 87% of *G. stearothermophilus* isolates and 78% of *M. thermoacetica* isolates belonged to 10 and 6 main groups, respectively. In each group and between groups, no correlation was found between strains and the nature of food product or the canneries involved or the year of isolation. Physiological studies were performed on 20 isolates for each species. Minimal and maximal growth temperatures were 40°C and 70°C for *G. stearothermophilus* and 45°C and 65°C for *M. thermoacetica*. Growth at different pH showed an acido-tolerant character for *M. thermoacetica* with 50% of isolates growing at pH 4.5 while no growth was obtained for *G. stearothermophilus* under pH 5.0. Sporulation efficiency (measured by microscopic observation) was very poor during 3 weeks in extremes conditions, except for one strain. Sporulation study is still running for *M. thermoacetica*. Low differences were obtained in heat resistance of spores produced in optimal conditions for the 20 isolates of *G. stearothermophilus* (0.2 min $\leq$D 120°C $\leq$ 2 min).

Significance: An important intraspecies diversity was shown for both species and further work seems necessary to establish a correlation between isolates present in canned foods before heat treatment and the ones responsible for canned foods spoilage. Data about growth and sporulation will allow us to give best recommendations concerning critical control points relevant for a vegetable processing line.

Keywords: *genetic diversity, growth, temperature, pH, sporulation, heat resistance*